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Review article

Challenging *Mycobacterium tuberculosis* dormancy mechanisms and their immunodiagnostic potential

Alexandre Silva Chaves, Michele Fernandes Rodrigues, Ana Márcia Menezes Mattos, Henrique Couto Teixeira *

Department of Parasitology, Microbiology and Immunology, Universidade Federal de Juiz de Fora, Juiz de Fora, MG, Brazil

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ABSTRACT

Mycobacterium tuberculosis is the etiologic agent of tuberculosis, one of the world's greatest cause of morbidity and mortality due to infectious disease. Many evolutionary mechanisms have contributed to its high level of adaptation as a host pathogen. Prior to become dormant, a group of about 50 genes related to metabolic changes are transcribed by the DosR regulon, one of the most complex and important systems of host-pathogen interaction. This genetic mechanism allows the mycobacteria to persist during long time periods, establishing the so-called latent infection. Even in the presence of a competent immune response, the host cannot eliminate the pathogen, only managing to keep it surrounded by an unfavorable microenvironment for its growth. However, conditions such as immunosuppression may reestablish optimal conditions for bacterial growth, culminating in the onset of active disease. The interactions between the pathogen and its host are still not completely elucidated. Nonetheless, many studies are being carried out in order to clarify this complex relationship, thus creating new possibilities for patient approach and laboratory screening.

Introduction

Mycobacterium tuberculosis is the causative agent of tuberculosis (TB), an infectious disease that remains a major global health issue.[1] Each year millions of people are accounted for infection, ranking TB as the second leading cause of death among infectious diseases, after the human immunodeficiency virus (HIV) infection. In 2013, nine million new cases of TB were notified leading to 1.5 million deaths despite the availability of treatment.[1]

One of the hallmarks of *M. tuberculosis* is the ability to establish a latent infection, capable of long persistence in the host, even in the presence of a functional immune system.[2] This persistent subclinical infection is driven by a low number of bacilli, which are kept in check by the host's immune

* *Corresponding author at*: Department of Parasitology, Microbiology and Immunology, Institute of Biological Sciences, Federal University of Juiz de Fora, Juiz de Fora, Minas Gerais 36036-900, Brazil.
E-mail address: henrique.teixeira@ufjf.edu.br (H.C. Teixeira).
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system,[3] a situation thought to exist in approximately one-third of the world's population.[1] Of those individuals, 5–10% may develop clinical manifestations of active disease at some point in their lifetime.[4] Most TB cases occur within 2–5 years of the initial infection, especially in young children.[1] Malnutrition, tobacco smoke, indoor air pollution, alcoholism, silicosis, insulin dependent diabetes, renal failure, malignancy, HIV infection, and immune suppressive treatment, are considered risk factors for reactivation.[5]

An important component of a public health response against TB in high-income countries is the investigation of contacts of known TB patients. In contrast, in low- and middle-income countries this policy is still being implemented.[6] In terms of prevalence, 29.1% of the contacts in high-income countries are latently infected, while 1.4% have active disease.[7] In low- and middle-income realities, 51.5% of the contacts have latent TB infection (LTBI), with 3.1% of contacts developing overt disease.[7] Interestingly, in these settings, reactivation rates of up to 40% are observed in children under 2 years old, predominantly within the first year after infection.[8] Healthcare workers are another significant risk group especially in low-and middle-income countries. The prevalence of LTBI among healthcare workers is, on average, 54%, emphasizing the importance of TB control in hospitals.[9] However, there is no agreement on the most common place for LTBI transmission, in addition, there is no gold standard for its diagnosis, which generates inaccuracy and misinterpretations depending on the methodology chosen.[6,10]

Currently in use, but far from gold standard

There are two main immunological approaches for screening *M. tuberculosis* latently infected individuals: the tuberculin skin test (TST), which is an *in vivo* assay, and the interferon-γ release assays (IGRAs), which comprises two *ex vivo* methods.[11] For the execution of the TST, a determined amount of purified protein derivative (PPD) is injected *via* the intradermal route into the forearm of the patient. The PPD is a protein extract from the culture supernatants of *M. tuberculosis*. If the reaction is positive, there is an indication of infection. Regarding the IGRAs, there are two distinct tools, an enzyme-linked immunosorbent assay (ELISA)-based technique, and an enzyme-linked immunospot (ELISpot)-based method.[12] Their most popular commercial versions are QuantiFERON®-TB Gold In-Tube (QFN-GIT; Cellestis Ltd, Carnegie, Victoria, Australia); and T-SPOT®.TB (Oxford Immunotec Ltd, Memphis, Tennessee, USA), respectively.[13] In a general sense, both enzyme-linked assays verify the immunologic memory of the host.[12] While the ELISA-based test determines the concentration of IFN-γ in supernatants of *M. tuberculosis* antigen-stimulated cell cultures, the ELISpot-based assay allows a quantitative assessment of IFN-γ-secreting cells in response to *M. tuberculosis*-specific antigens.[11]

The sensitivity of the TST, which is the most widespread test, is extremely dependent on the patient's immunological status, being lower in immunocompromised patients.[13] There is growing evidence that the sensitivity of the IGRAs are higher than that of the TST; however, the lack of studies carried out in immunocompromised subjects and the impossibility of a clear differentiation between active disease and LTBI are major setbacks.[14] As another important consideration, the sensitivity of TST is also dependent on the choice of the cut-off value, thus, entangling with the specificity, which is affected by the cross-reactivity between *M. tuberculosis* antigens and environmental mycobacteria.[15] Various groups have performed reviews and meta-analysis of sensitivity and specificity values to help clinicians and agencies to develop guidelines toward the diagnosis of LTBI. According to Pai et al.,[16] the sensitivity of IGRAs scored a value of 70% for QFN-GIT and 90% for T-SPOT.TB, while TST reached a mark of 77%. Diel et al.[17] verified that IGRAs were more reliable for the identification of non-infected individuals, as compared to TST. The specificity for QFN-GIT, T-SPOT.TB, and TST were 99.4%, 98%, and 88.7%, respectively.[17] However, in high-incidence settings, a positive IGRA may not necessarily indicate TB, as much as a negative IGRA or negative TST cannot rule out the possibility of active disease.[16]

In high-income countries, in which low TB rates are found, LTBI screening is recommended in the subjects at increased risk of developing the disease as a matter of public health.[18] However the lack of clear cut-off values for serial testing and unclear interpretation make useful tools like the IGRAS still hard to be implemented in all realities.[10,18] Besides, the exact proportion of positive tests for LTBI and the number of patients that still have not received preventive chemotherapy is still unknown. Public health policies and clinical management for the identification and treatment of latently infected subjects would be improved with a better understanding of the nature of LTBI.[2]

The key mechanism for latent infection

For survival and persistence in extreme conditions, *M. tuberculosis* must be able to sense environmental signals and utilize them to trigger its adaptation machinery, then making its endurance possible in a new ambient.[19] Low oxygen tension, oxidative stress, and NO are factors that are frequently associated with the establishment and maintenance of LTBI.[20] In this context, the transcription factor Rv3133c, named dormancy survival regulator (DosR), directly coordinates the expression of approximately 50 genes, which altogether make up the regulon DosR, preparing the metabolic changes that will allow the mycobacteria to enter dormancy.[21,22]

Under aerobic conditions, the transcription factor PhoP (Rv0757) is responsible for the maintenance of basal levels of Rv3133c.[23] When hypoxia is set, Rv3133c may have more five-fold increase compared to its original values,[24] enabling the entire regulon to be induced.[23] Remarkably, the first cellular changes can occur under low concentrations of Rv3133c. So, the first genes to be transcribed are those related to protein stability and homeostatic regulation, such as *hspx* (*rv2031*) and *rv1738*, respectively, preparing the cell for further metabolic changes.[25]

Though strictly aerobic, *M. tuberculosis* may face low oxygen levels during the course of infection, typically in late granulomas, which are characteristically avascular, inflammatory, and necrotic. Those conditions have been demonstrated as hypoxic.[26] *In vivo* models that mimic hypoxic conditions